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QuantiFERON Monitor® (QFM®) ELISA Package Insert ▽Σ 2 x 96

The whole blood IFN-γ test measuring responses to innate and adaptive immune stimulants

Version 1

IVD For in vitro diagnostic use

CE

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Contents

Intended Use

The QuantiFERON Monitor assay (QFM) is an in vitro diagnostic test intended to detect cell-mediated immune function through the measurement of interferon gamma (IFN-γ) in plasma by enzyme-linked immunosorbent assay (ELISA) following incubation of heparinized whole blood with innate and adaptive immune response stimulants. The assay is used to detect cell mediated immune response in the immunosuppressed solid organ transplant population.

QFM is intended for use in conjunction with risk assessment and other medical and diagnostic evaluations.

Summary and Explanation of the Test

Immunodeficiency is characterized by a reduced ability to effectively mount an immune response. This compromised or absent response can result from a primary or acquired (secondary) immunodeficiency (1).

Primary immunodeficiencies are genetically inherited and characterized by deficiencies of distinct components of the adaptive or innate immune system (1). Nonetheless, most immunodeficiencies are acquired (secondary) and can be induced by pathogenic agents, drugs (such as immunosuppressive treatment following organ transplantation), disease conditions (such as cancer, e.g., leukemia and lymphoma), or by environmental contaminants (1).

The molecular basis of immunodeficiency is diverse; however, cell-mediated immunity plays a key role in inducing many of the observed clinical manifestations. At present, diagnosis and management of immunodeficiency syndromes is dependent on the causal agent (2, 3).

For example, ad hoc management is the norm in monitoring the cellular immunodeficiency status of subjects who have undergone solid organ transplants (SOTs) and are receiving medications to suppress their immune system. The status of the subject's immune response is generally measured by monitoring pharmacologic drug levels and clinical/pathological evaluation of graft function (2, 3).

A number of T-cell function tests measure cell-mediated immunity to mitogens such as phytohemagglutinin (PHA), pokeweed mitogen, and concanavalin A (ConA); however, these only measure the functional ability of T cells and are a subset of cells involved in cell-mediated immunity. It has become increasingly evident that innate immune mechanisms contribute greatly to host defense, either through acting alone or by enhancing specific T-cell responses. Therefore, the functional responses of innate (natural killer [NK] cell) and adaptive (T cell)

immune cells together form a more comprehensive analysis of cell-mediated immunity (2, 3).

QFM is an in vitro diagnostic test that uses a combination of stimulants (in the form of a LyoSphere™ pellet) that specifically stimulate different cell types involved in both the innate and adaptive immune systems. The functional immune status of a subject is assessed by measuring the response to stimulation of the innate and adaptive immune system with Toll Like Receptor (TLR) and T-cell receptor (TCR) agonists, respectively. The detection of Interferon-gamma (IFN-γ) by ELISA provides both a qualitative and quantitative measure of cell-mediated immune function.

Principles of the assay

The QFM assay uses lyophilized stimulants (QFM LyoSpheres™), which are added to heparinized whole blood. Incubation of the blood occurs for 16 to 24 hours, after which, plasma is harvested and tested for the presence of IFN-γ produced in response to the stimulants.

The QFM test is performed in stages. First, whole blood is collected into the QFM Blood Collection Tube. Next, a QFM LyoSphere is added to the tube, which is then incubated at 37°C as soon as possible and within 8 hours of collection. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-γ (reported in International Units per ml; IU/ml) measured by ELISA and compared to a range of expected values to characterize the immune response of the subject.

QFM is an assay that provides both a qualitative and quantitative measure of immune function. QFM results may not directly quantify the level of immune suppression.

The amount of IFN-γ in plasma samples may often be above the upper limits of most ELISA readers, even when individuals are moderately immunosuppressed. It is recommended that plasma samples are diluted 1 in 10 and/or 1 in 100 in Green Diluent and assayed in the ELISA together with undiluted plasma.

Note: The threshold of the QFM assay may vary depending on a subject's level of immunosuppression and individual transplant settings.

See "Interpretation of Results" on page 23 of this package insert for an outline of how QFM results are interpreted.

Time required to perform assay

The time required to perform the QFM assay is estimated below. The time of testing multiple samples when batched is also indicated.

37°C incubation of blood tubes: 16 to 24 hours

ELISA: Approximately 3 hours for one ELISA plate (up to 88 samples)

<1 hour labor

Add 10 to 15 minutes for each extra plate

Components and Storage

QuantiFERON Monitor LyoSpheres	
Catalog no.	0650-0701
Number of preps	10
QuantiFERON Monitor LyoSpheres	10 vials
QuantiFERON Monitor LyoSpheres Package Insert	1
QuantiFERON Monitor Blood Collection Tubes	
Catalog no.	0650-0101
Number of preps	100
QuantiFERON Monitor Blood Collection Tubes (white cap, white ring)	100 tubes
QuantiFERON Monitor Blood Collection Tubes Package Insert	1

QuantiFERON Monitor 2 Plate Kit ELISA components	2-plate kit ELISA
Catalog no.	0650-0201
Microplate Strips,12 x 8 wells (coated with murine anti-human IFN-γ monoclonal antibody)	2 sets 12 x 8-well Microplate Strips
IFN-γ Standard, lyophilized (contains recombinant human IFN-γ, bovine casein, 0.01% w/v Thimerosal)	1 x vial (8 IU/ml when reconstituted)
Green Diluent (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)	1 x 30 ml vial
Conjugate 100x Concentrate, lyophilized (murine anti-human IFN-γ HRP, contains 0.01% w/v Thimerosal)	1 x 0.3 ml, when reconstituted
Wash Buffer 20x Concentrate (pH 7.2, contains 0.05% v/v ProClin® 300)	1 x 100 ml
Enzyme Substrate Solution (contains H_2O_2, 3,3′, 5,5′ Tetramethylbenzidine)	1 x 30 ml
Enzyme Stopping Solution (contains 0.5 M H_2SO_4)*	1 x 15 ml
QuantiFERON Monitor ELISA package insert	1

* Contains sulfuric acid. See page 11 for precautions.

Materials required but not provided

- 37°C incubator*; CO_2 not required.
- Calibrated variable-volume pipets*
- Calibrated multichannel pipet† capable of delivering 50 µl and 100 µl with disposable tips
- Microplate shaker†
- Deionized or distilled water, 2 liters
- Microplate washer (automated washer recommended)
- Microplate reader† fitted with 450 nm filter and 620 nm to 650 nm reference filter
- Graduated cylinder (measuring cylinder)
- Low-lint absorbent towels
- Plate lid

Storage and handling

Blood collection tubes

Store QFM Blood Collection Tubes at 4°C to 25°C. QFM Blood Collection Tubes should be between 17°C–25°C at time of blood filling and mixing.

LyoSpheres

Store QFM LyoSpheres at 2°C to 8°C.

ELISA kit reagents

Store ELISA kit reagents at 2°C to 8°C.

Always protect Enzyme Substrate Solution from direct sunlight.

* Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Reconstituted and unused ELISA reagents

For instructions on how to reconstitute the ELISA reagents, please see "Stage 2 — IFN-γ ELISA", page 17.

- The reconstituted kit standard may be kept for up to 3 months if stored at 2°C to 8°C.

  Note the date on which the kit standard was reconstituted.

- Once reconstituted, unused Conjugate 100x Concentrate must be returned to storage at 2°C to 8°C and must be used within 3 months.

  Note the date on which the conjugate was reconstituted.

- Working strength conjugate must be used within 6 hours of preparation (see Table 1).
- Working strength wash buffer may be stored at room temperature (22°C ± 5°C) for up to 2 weeks.

Warnings and Precautions

For in vitro diagnostic use

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Warnings

- QFM is an assay that provides both a qualitative and quantitative measure of immune function. QFM results may not directly quantify the level of immune suppression.
- Results of the QFM assay should be used in conjunction with clinical presentation, medical history, and other clinical indicators when establishing the immune status of a patient.
- The threshold of the QFM assay may vary depending on a subject's level of immunosuppression and individual transplant settings.

Precautions

For in vitro diagnostic use only.

CAUTION: Handle human blood and plasma as if potentially infectious. Observe relevant blood and blood product handling guidelines. Dispose of samples and materials in contact with blood or blood products in accordance with federal, state, and local regulations.

The following hazards and precautionary statements apply to components of the QuantiFERON Monitor ELISA.

Hazard Statements

QuantiFERON Enzyme Stopping Solution

Contains: sulfuric acid. Warning! May be corrosive to metals. Causes skin irritation. Causes serious eye irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Enzyme Substrate Solution

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Green Diluent

Contains: trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo)pyrazole-3-carboxylate. Contains: tartrazine. Warning! May cause an allergic skin reaction. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Wash Buffer 20x Concentrate

Contains: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H - isothiazol-3-one (3:1). Harmful to aquatic life with long lasting effects. Avoid release to the environment.

Further information

Safety Data Sheets: www.qiagen.com/safety

- Deviations from the *QuantiFERON Monitor (QFM) ELISA Package Insert* may lead to erroneous results. Read the instructions carefully before use.

- **Important:** Inspect vials prior to use. Do not use Conjugate, IFN-γ Standard or QFM LyoSphere vials that show signs of damage or if the rubber seal has been compromised. Do not handle broken vials. Take the appropriate safety precautions to dispose of vials safely.
  Recommendation: Use a vial de-crimper to open the Conjugate, IFN-γ Standard or QFM LyoSphere vials to minimize risk of injury from the metal crimp cap.
- Do not use the ELISA kit if any reagent bottle shows signs of damage or leakage prior to use.
- Do not mix or use the Microplate Strips, IFN-γ Standard, Green Diluent, or Conjugate 100x Concentrate from different QFM ELISA batches. Other reagents (Wash Buffer 20x Concentrate, Enzyme Substrate Solution, and Enzyme Stopping Solution) can be interchanged among kits, providing the reagents are within their expiration periods and lot details are recorded.
- Discard unused reagents and biological samples in accordance with local and national safety and environmental regulations.
- Do not use the QFM Blood Collection Tubes, QFM LyoSpheres, or QFM ELISA after the expiration date.
- Make sure that laboratory equipment has been calibrated/validated for use.

Specimen Collection and Handling

The QFM assay should only be performed using whole blood collected in either a lithium heparin blood collection tube or directly into a QFM Blood Collection Tube; 1 ml whole blood is required per test. Blood collection tubes must be labeled appropriately and include the time of blood collection.

Important: Both the stimulation of QFM blood samples (i.e., the addition of a QFM LyoSphere to a 1 ml blood aliquot) and their subsequent incubation at 37°C must occur within 8 hours of blood collection.

Prior to incubation, maintain the blood samples at room temperature (22°C ± 5°C).

The following procedures should be followed for optimal results:

1. Label tubes appropriately.

   Make sure each QFM Blood Collection Tube is labeled appropriately with the subject's details and time of blood collection.

2. For each subject collect 1 ml of blood by venipuncture directly into a QFM Blood Collection Tube. A trained phlebotomist should perform this procedure.

   Important note: Tubes should be between 17°C to 25°C at the time of blood filling.

   QFM Blood Collection Tubes can be used up to an altitude of 810 meters above sea level.

   As 1 ml tubes draw blood relatively slowly, keep the tube on the needle for 2–3 seconds once the tube appears to have completed filling. This will ensure that the correct volume is drawn.

   The black mark on the side of the QFM Blood Collection Tube label indicates a 1 ml fill volume. QFM Blood Collection Tubes are manufactured to draw 1 ml ± 10% and perform optimally within this range. If the level of blood is outside the range of the indicator line, a new blood sample should be obtained.

   If a "butterfly" needle is used to collect blood, use a "purge" blood collection tube to make sure that the tubing is filled with blood prior to the QFM Blood Collection Tubes being used.

   If using QFM Blood Collection Tubes at an altitude higher than 810 meters, or if low blood volume occurs, collect blood using a syringe and immediately transfer 1 ml of blood to the QFM Blood Collection Tube. For safety reasons, this is best performed by removing the syringe needle, ensuring appropriate safety procedures, removing the cap from the QFM

Blood Collection Tube and adding 1 ml of blood (to the center of the black mark on the side of the tube label). Replace the cap securely and mix as described below.

If you use a tourniquet, loosen the tourniquet as soon as the needle is inserted into the vein to avoid variations in pressure which could impact blood volume.

Alternatively, blood may be collected into a generic blood collection tube containing lithium heparin as the anticoagulant and then transferred to a QFM Blood Collection Tube. Only use lithium heparin as a blood anticoagulant because other anticoagulants interfere with the assay. Fill a blood collection tube (minimum volume of 3 ml) and gently mix by inverting the tube several times to dissolve the heparin. Maintain the blood at room temperature (22°C ± 5°C) before transfer to QFM Blood Collection Tubes for stimulation with a QFM LyoSphere. Make sure that the blood is thoroughly mixed by gentle inversion immediately prior to dispensing. Dispense a 1 ml aliquot of blood into a QFM Blood Collection Tube. Perform dispensing aseptically, ensuring appropriate safety procedures are followed while removing the cap from the QFM Blood Collection Tube and adding 1 ml of blood (to center of the black mark on the side of the tube label). Replace the tube caps securely and mix as described below.

3. Immediately after filling the tubes, invert the tube gently several times to dissolve the heparin.

   Important: Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

4. Just before use, equilibrate QFM LyoSpheres to room temperature (22°C ± 5°C).
5. Aseptically add one QFM LyoSphere to 1 ml of blood.

   Uncap the blood collection tube.

   Tap the QFM LyoSphere vial gently on a hard surface to ensure the QFM LyoSphere is located at the bottom of the vial. Uncap the QFM LyoSphere vial by first removing the metal crimp cap and then the rubber stopper.

   Carefully drop the QFM LyoSphere into the 1 ml blood sample by aligning the lip of the glass vial to the lip of the QFM Blood Collection Tube and then invert the vial gently to transfer the QFM LyoSphere into the QFM Blood Collection Tube (see Figure 1).

   Important: If the QFM LyoSphere falls outside the QFM Blood Collection Tube, discard it and open another QFM LyoSphere vial.

Important: Do not leave the QFM LyoSphere vial open for extended periods of time. The QFM LyoSphere should be added to blood as soon as you uncap the vial.

If QFM LyoSpheres are being added to blood that has been collected into the QFM Blood Collection Tube, ensure that the tube caps are returned to the correct samples.

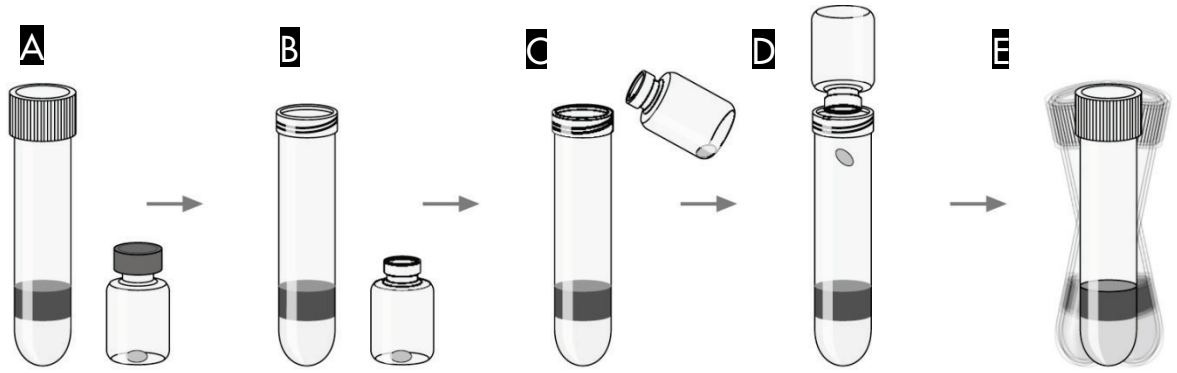


Figure 1. Adding QFM LyoSphere. A QFM Blood Collection Tube and QFM LyoSphere vial. B Remove the cap from the QFM Blood Collection Tube and remove the metal crimp and rubber stopper from QFM LyoSphere vial. C Immediately add the QFM LyoSphere to the blood by aligning the lip of the glass vial to the lip of the collection tube. D Next, invert the vial gently to transfer the LyoSphere into the collection tube. E Recap the QFM Blood Collection Tube and shake 5–10 times.

6. Cap the QFM Blood Collection Tube and shake it 5–10 times, just firmly enough to make sure that the QFM LyoSphere has completely dissolved.

   If a QFM LyoSphere adheres to the inner tube surface, it can be dissolved by coating the LyoSphere in blood while inverting the tube.

   Make sure the tube is capped once the QFM LyoSphere has been added to prevent the accidental addition of a second LyoSphere to the same tube.

   Note: Because the QFM LyoSphere is white, it will no longer be visible in blood once it has dissolved.

   Important: Overly vigorous shaking may cause gel disruption and could lead to aberrant results.

7. After adding and dissolving the QFM LyoSphere, QFM Blood Collection Tubes must be transferred to a 37°C ± 1°C incubator as soon as possible and within 8 hours of blood collection.

Directions for Use

Stage 1 — incubation of blood and harvesting of plasma

Materials provided

- QFM Blood Collection Tubes (refer to "Components and Storage", page 6)

Materials required (but not provided)

- Refer to "Materials required but not provided", page 8

Procedure

1. Incubate QFM Blood Collection Tubes containing 1 ml blood aliquots with QFM LyoSphere UPRIGHT at 37°C ± 1°C for 16 to 24 hours.

   Note: The incubator does not require CO_2 or humidification.

   After incubation, QFM Blood Collection Tubes may be held between 4°C–27°C for up to 3 days prior to centrifugation.

2. After incubation, harvesting of plasma is facilitated by centrifuging the QFM Blood Collection Tubes for 15 minutes at 2000 to 3000 x *g* (RCF). The gel plug will separate the cells from the plasma. If this does not occur, re-centrifuge the tubes.

   It is possible to harvest the plasma without centrifugation; however, additional care is required to remove the plasma without disturbing the cells.

3. Plasma samples should only be harvested using a pipet.

   Important: After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.

   Plasma samples can be loaded directly from centrifuged QFM Blood Collection Tubes into the QFM ELISA plate, including when automated ELISA workstations are used.

   Plasma samples can be stored for up to 28 days at 2°C to 8°C, or if harvested, below –20°C for extended periods. Aliquots of harvested plasma samples must be sealed prior to storage.

   If harvesting plasma samples, harvest at least 150 µl of plasma to allow for repeat testing if required.

   The amount of IFN-γ in plasma samples may often be above the upper limits of most ELISA readers, even when individuals are moderately immunosuppressed. It is recommended that plasma samples are diluted

1:10 and/or 1:100 in Green Diluent and assayed in the ELISA together with undiluted plasma (see Stage 2 – performing IFN-γ ELISA).

Stage 2 — IFN-γ ELISA

Materials provided

- QuantiFERON Monitor 2 Plate Kit ELISA (refer to "Components and Storage", page 6)

Materials required (but not provided)

- Refer to "Materials required but not provided", page 8

Preparation

IFN-γ in plasma may often be above the upper limits of most ELISA readers, even when individuals are moderately immunosuppressed. Recommended: dilute plasma samples 1:10 and/or 1:100 in Green Diluent and assay in the ELISA together with undiluted plasma.

In situations where a patient may be heavily immunosuppressed, preparing and assaying only an undiluted plasma sample may be sufficient to obtain a quantitative result.

Note: Sample results that are within the range of the QFM ELISA (i.e., up to 10 IU/ml) should be used for Interpretation of Results. The lowest dilution that generates a result within the range of the QFM ELISA should be used as the reported result (taking the dilution factor into account) if undiluted plasma is above the range of the QFM ELISA.

Procedure

1. All plasma samples and reagents, except for Conjugate 100x Concentrate, must be brought to room temperature (22°C ± 5°C) before use. Allow at least 60 minutes for equilibration.
2. Remove strips that are not required from the microplate frame, reseal in the foil pouch, and return to the refrigerator for storage until needed.

   Allow at least one strip for the QFM standards and sufficient strips for the number of subjects being tested. After use, retain frame and lid for use with remaining strips.
3. Reconstitute the lyophilized IFN-γ Standard with the volume of deionized or distilled water indicated on the label of the Standard vial. Mix gently to minimize frothing and ensure complete solubilization. Reconstitution of the

Standard with the stated volume will produce a solution with a concentration of 8.0 IU/ml.

Important: The reconstitution volume of the IFN-γ Standard differs between batches. Consult the label of the standard vial to ensure that you use the correct volume of deionized or distilled water.

Use the reconstituted kit standard to produce a 1 in 2 dilution followed by a 1 in 4 dilution series of IFN-γ in Green Diluent (GD) (see Figure 2). S1 (Standard 1) contains 4.0 IU/ml, S2 (Standard 2) contains 1.0 IU/ml, S3 (Standard 3) contains 0.25 IU/ml, and S4 (Standard 4) contains 0 IU/ml (GD alone). The standards should be assayed in duplicate. Prepare fresh dilutions of the kit standard for each ELISA session.

Recommended procedure for duplicate standards
a. Label 4 tubes "S1", "S2", "S3", "S4".
b. Add 150 µl of GD to SI, S2, S3, and S4.
c. Add 150 µl of the kit standard to S1 and mix thoroughly.
d. Transfer 50 µl from S1 to S2 and mix thoroughly.
e. Transfer 50 µl from S2 to S3 and mix thoroughly.
f. Green Diluent (GD) alone serves as the zero standard (S4).

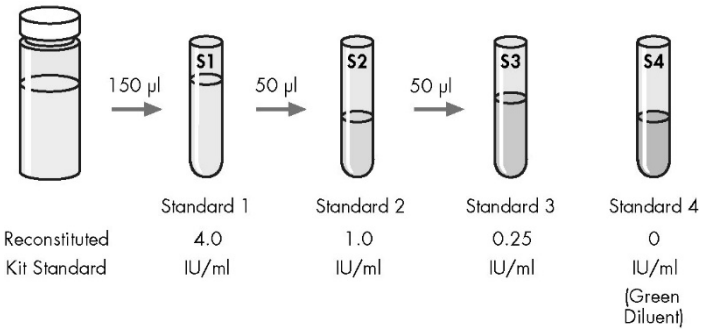


Figure 2. Preparation of standard curve.

4. Reconstitute lyophilized Conjugate 100x Concentrate with 0.3 ml of deionized or distilled water. Mix gently to minimize frothing and ensure complete solubilization of the conjugate.

Working strength conjugate is prepared by diluting the required amount of reconstituted Conjugate 100x Concentrate in Green Diluent (Table 1. Conjugate Preparation). Return any unused Conjugate 100x Concentrate to 2°C to 8°C immediately after use. Use only Green Diluent.

Table 1. Conjugate Preparation

Number of strips	Volume of Conjugate 100x Concentrate	Volume of Green Diluent
2	10 µl	1.0 ml
3	15 µl	1.5 ml
4	20 µl	2.0 ml
5	25 µl	2.5 ml
6	30 µl	3.0 ml
7	35 µl	3.5 ml
8	40 µl	4.0 ml
9	45 µl	4.5 ml
10	50 µl	5.0 ml
11	55 µl	5.5 ml
12	60 µl	6.0 ml

5. For plasma samples harvested from blood collection tubes and subsequently stored or frozen, mix samples before addition to the ELISA well.

   Important: If plasma samples are added directly from centrifuged QFM tubes, any mixing of the plasma should be avoided. At all times, take care not to disturb material on the surface of the gel.

6. Recommended: Dilute plasma samples 1:10.
   - Add 90 µl Green Diluent (GD) to a tube labeled with patient details and "1:10".
   - Then, add 10 µl of mixed plasma samples (see step 5 for details on mixed plasma samples vs. those added directly from centrifuged QFM tubes).
   - Mix thoroughly by pipet, minimizing frothing.

7. Recommended: Dilute 1:100 plasma samples.
   - Prepare a 1:10 dilution (see step 6 above).
   - Add 90 µl of Green Diluent to a tube labeled with patient details and "1:100".
   - Add 10 µl of the 1:10 dilution.
   - Mix thoroughly by pipet, minimizing frothing.

   Recommended: Test the following samples in parallel, and in this order:
   - Undiluted, 1:10, 1:100

   The following subject sample options are also supported by the QFM Analysis Software:
   - Undiluted
   - 1:10
   - 1:100
   - 1:10, 1:100
   - Undiluted, 1:10
8. Add 50 µl of freshly prepared, working strength conjugate to the required ELISA wells using a multichannel pipet.
9. Add 50 µl of test plasma sample to appropriate wells using a multichannel pipet. Then add 50 µl of each of standards 1 to 4. Assay the standards in duplicate.
10. Cover each plate and mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute. Avoid splashing.
11. Incubate at room temperature (22°C ± 5°C) for 120 ± 5 minutes.

    Plates should not be exposed to direct sunlight during incubation.
12. During incubation, dilute 1 part Wash Buffer 20x Concentrate with 19 parts deionized or distilled water and mix thoroughly. Sufficient Wash Buffer 20x Concentrate has been provided to prepare 2 liters of working strength wash buffer.

    Wash wells with 400 µl of working strength wash buffer for at least 6 cycles in a microplate washer. An automated plate washer is recommended.

    Thorough washing is very important to the performance of the assay. Make sure each well is completely filled with wash buffer for each wash cycle. Recommended: Soak wells for a period of at least 5 seconds between each cycle for best results.

Add standard laboratory disinfectant to the effluent reservoir and follow established procedures for the decontamination of potentially infectious material.

13. Tap plates face down on absorbent, low-lint towel to remove residual wash buffer. Add 100 µl of Enzyme Substrate Solution to each well, cover each plate, and mix thoroughly using a microplate shaker.
14. Incubate at room temperature (22°C ± 5°C) for 30 minutes.

    Plates should not be exposed to direct sunlight during incubation.
15. Following incubation, add 50 µl of Enzyme Stopping Solution to each well and mix thoroughly using a microplate shaker.

    Enzyme Stopping Solution should be added to wells in the same order and at approximately the same speed as used when adding the Enzyme Substrate Solution in step 13.
16. Measure the Optical Density (OD) within 5 minutes of stopping the reaction using a microplate reader fitted with a 450 nm filter as well as a 620 nm to 650 nm reference filter. OD values are used to calculate results.

Calculations and Test Interpretation

QuantiFERON Monitor Analysis Software is used to analyze raw data and calculate results. It is available from www.QuantiFERON.com. Please make sure that the most current version of the QuantiFERON Monitor Analysis Software is used.

The software performs a quality control assessment of the assay, generates a standard curve, and provides a test result for each subject, as detailed in the Interpretation of Results section.

If undiluted plasma is above the upper range (i.e., >10 IU/ml) of the QFM ELISA, the QuantiFERON Monitor Analysis Software reports the lowest dilution that generates a result within the range of the QFM ELISA, taking the dilution factor into account.

As an alternative to using the QuantiFERON Monitor Analysis Software, results can be determined according to the following method.

Generation of standard curve

(If QuantiFERON Monitor Analysis Software is not used)

Determine the mean OD values of the kit standard replicates on each plate.

Construct a $\log_{(e)}$-$\log_{(e)}$ standard curve by plotting the $\log_{(e)}$ of the mean OD (y-axis) against the $\log_{(e)}$ of the IFN-γ concentration of the standards in IU/ml (x-axis), omitting the zero standard from these calculations. Calculate the line of best fit for the standard curve by regression analysis.

Use the standard curve to determine the IFN-γ concentration (IU/ml) for each of the test plasma samples, using the OD value of each sample.

These calculations can be performed using software packages available with microplate readers and standard spreadsheet or statistical software (such as Microsoft® Excel® software). It is recommended that these packages be used to calculate the regression analysis, the coefficient of variation (%CV) for the standards, and the correlation coefficient (*r*) of the standard curve.

The reported result should be taken from the lowest dilution that generates a result within the range of the QFM ELISA (taking the dilution factor into account) if undiluted plasma is above the range of the QFM ELISA.

Quality control of test

The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the ELISA to be valid:

- The mean OD value for Standard 1 must be ≥0.600.
- The %CV for Standard 1 and Standard 2 replicate OD values must be ≤15%.
- Replicate OD values for Standard 3 and Standard 4 must not vary by more than 0.040 optical density units from their mean.
- The correlation coefficient (*r*) calculated from the mean absorbance values of the standards must be ≥0.98.

The QuantiFERON Monitor Analysis Software calculates and reports these quality control parameters.

If the above criteria are not met, the run is invalid and must be repeated.

The mean OD value for the zero standard (Green Diluent) should be ≤0.150. If the mean OD value is >0.150, the plate washing procedure should be investigated.

Interpretation of results

QFM results are interpreted depending on IFN-γ response to innate and adaptive immune stimulants. The QFM assay provides both a qualitative and quantitative measure of immune function. QFM results may not directly quantify the level of immune suppression.

Important: When establishing the immune status of a subject, the measured IFN-γ level should be used in conjunction with clinical presentation, medical history, and other diagnostic evaluations (Table 2). The threshold of the QFM test may vary depending on a subject's level of immunosuppression and the individual transplant settings.

Table 2. Interpretation of results

QFM result IFN-γ (IU/ml)	Classification	Interpretation
<15	Low	Subject has low IFN-γ response to innate and adaptive immune stimulants
15–1000	Moderate	Subject has moderate IFN-γ response to innate and adaptive immune stimulants
>1000	High	Subject has high IFN-γ response to innate and adaptive immune stimulants

If the measured IFN-γ level of an undiluted plasma sample is less than 0.1 IU/ml:

- Make sure that the QFM LyoSphere was added to the blood sample and the tube was incubated as directed in this package insert.
- Make sure that the IFN-γ result corresponds with the current clinical status of the subject.

If technical issues are suspected with the collection or handling of blood samples, repeat the entire QFM assay with a new blood sample. Repeat the ELISA testing of stimulated plasma samples if it is suspected that the original test deviated from the procedure described in this package insert (see Quality Control of Test section for details).

The physician may wish to repeat the test if results are inconsistent with the current clinical status of the subject.

Limitations

Results from QFM testing must be used in conjunction with each individual's clinical history, current medical status, and other diagnostic evaluations. Laboratories may choose to establish their own ranges for the assay.

Laboratories may also choose to run an external control specimen collected from a healthy subject in parallel with patient specimens.

Unreliable or inaccurate results may occur due to:

- Incorrect blood anticoagulant — use only lithium heparin because other anticoagulants interfere with the assay.
- Deviations from the procedure described in this package insert.
- Excessive levels of circulating IFN-γ or presence of heterophile antibodies.
- Longer than 8 hours between drawing the blood specimen and incubation at 37°C.
- Underfilling or overfilling QFM blood tubes outside of the 0.9 to 1.1 ml range.

Performance Characteristics

Clinical studies

Two clinical studies were conducted to evaluate the responses of apparently healthy individuals (n=114) vs. transplant recipients (n=30). Of the transplant recipients, 18 were in the early posttransplant cohort (Early Post-Tx, within 3 months after transplant) and 12 were in the late posttransplant or stable cohort (Late Post-Tx, >12 months after transplant).

- Samples were collected at up to 5 time points from each individual in Early Post-Tx (3 months posttranplant cohort, n=64 samples).
- Samples were collected 1 time from each individual in Late Post-Tx (late posttransplant cohort, n=12 samples)
- Samples were collected 1 time from each individual in the apparently healthy cohort (n=114)

Responses to QFM ranged between low and moderate in both Early Post-Tx samples and Late Post-Tx samples. Early Post-Tx had a higher percentage (93.8%) of responses within the low range, and a lower percentage of responses (6.3%) within the moderate range in comparison to the responses from the Late Post-Tx, with 25% of responses in the low range and 66.7% in the

moderate range (Table 3). No responses of Early Post-Tx were in the high-response range, while only 1 (8.3%) response in Late Post-Tx samples was in the high-response range. QFM responses in the apparently healthy cohort were mainly in the moderate range (83.3%) and high-response range (15.8%)(Table 3).

Table 3. QFM response range in apparently healthy subjects vs. transplant recipients

IFN-γ (IU/ml)	Results category	Early Post-Tx %* 95% CI n	Late Post-Tx %* 95% CI n	Apparently Healthy %* 95% CI n	Total results
<15	Low	93.8% 85.0–97.5 n=60	25.0% 8.9–53.2 n=3	0.9% 0.2–4.8 n=1	64
15–1000	Moderate	6.3% 2.5–15.0 n=4	66.7% 39.1–86.2 n=8	83.3% 75.4–89.1 n=95	107
>1000	High	0.0% 0–5.7 n=0	8.3% 1.5–35.4 n=1	15.8% 10.2–23.6 n=18	19
Total samples		64	12	114	190

* Percentages indicate the proportion of samples within each donor cohort that fall within the particular response range.

Expected values

The distribution of IFN-γ responses to QFM in early posttransplant patients (up to 3 months posttransplant) was determined from 64 samples collected from 18 transplant recipients using the QFM ELISA (Figure 3).

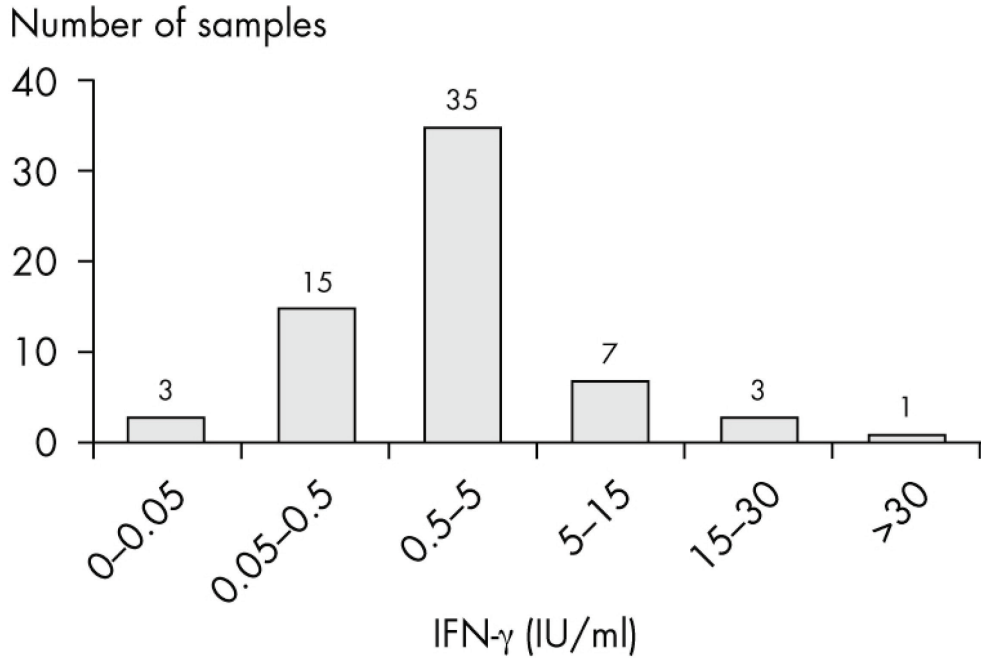


Figure 3. Distribution of QFM IFN-γ responses in early posttransplant patients (n=64; median=1.5 IU/ml).

The distribution of IFN-γ responses to QFM in late posttransplant patients (>12 months posttransplant) was determined from 12 samples using the QFM ELISA (Figure 4).

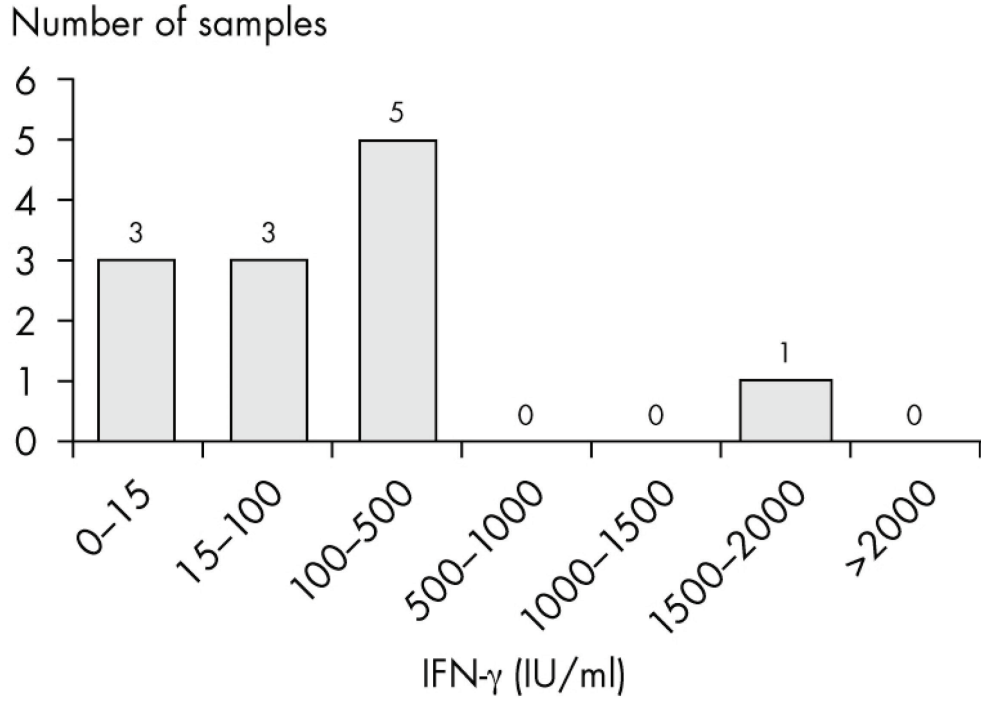


Figure 4. Distribution of QFM IFN-γ responses in late posttransplant patients (n=12; median=98.8 IU/ml).

The distribution of IFN-γ responses to QuantiFERON Monitor in apparently healthy subjects was determined from 114 samples using the QFM ELISA (Figure 5).

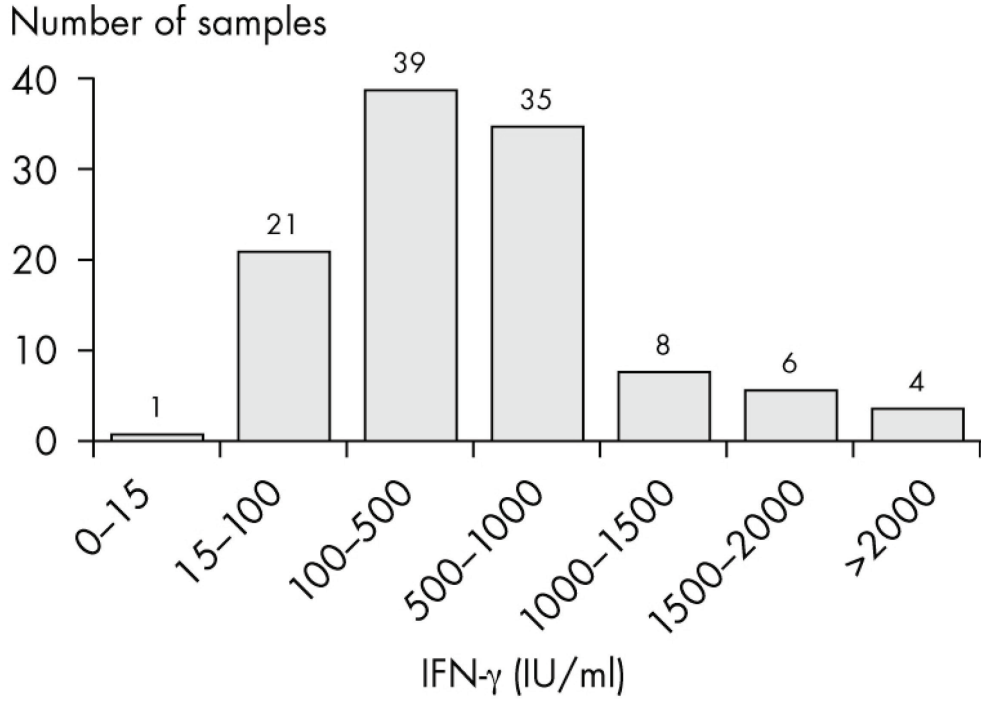


Figure 5. Distribution of QFM IFN-γ responses in apparently healthy subjects (n=114; median=400.5 IU/ml).

QFM responses in solid organ transplant patients

QFM was evaluated in an observational, cross-sectional study of solid organ transplant patients (4). The study included: 212 healthy subjects with a subgroup of 30 age- and sex-matched controls, 30 pretransplant patients, 18 early posttransplant patients (66 samples; median time posttransplant=21 days), and 11 late posttransplant patients (median time posttransplant=2290 days). Mean IFN-γ production was 555.2 IU/ml in healthy controls, and 614.6 IU/ml in age- and sex- matched controls. Mean IFN-γ production was shown to be significantly lower in both pretransplant (IFN-γ=89.3 IU/ml) and early posttransplant (IFN-γ=3.76 IU/ml) patients compared to the age- and sex-matched controls ($p<0.001$). Restoration of immune function in the late posttransplant patients (mean IFN-γ=256.1 IU/ml) was observed and demonstrated to be significantly greater than early posttransplant patients ($p<0.05$). This study indicates QFM may be used to assess cell mediated immune function in the immunosuppressed solid organ transplant population.

Assay performance characteristics

The QFM ELISA has been demonstrated to be linear by placing 5 replicates of 11 plasma pools of known IFN-γ concentrations randomly on the ELISA plate. The linear regression line has a slope of 1.002 ± 0.011 and a correlation coefficient of 0.99 (Figure 6).

The limit of detection of the QFM ELISA is 0.065 IU/ml and there is no evidence of a high-dose hook (prozone) effect with concentrations of IFN-γ up to 10,000 IU/ml.

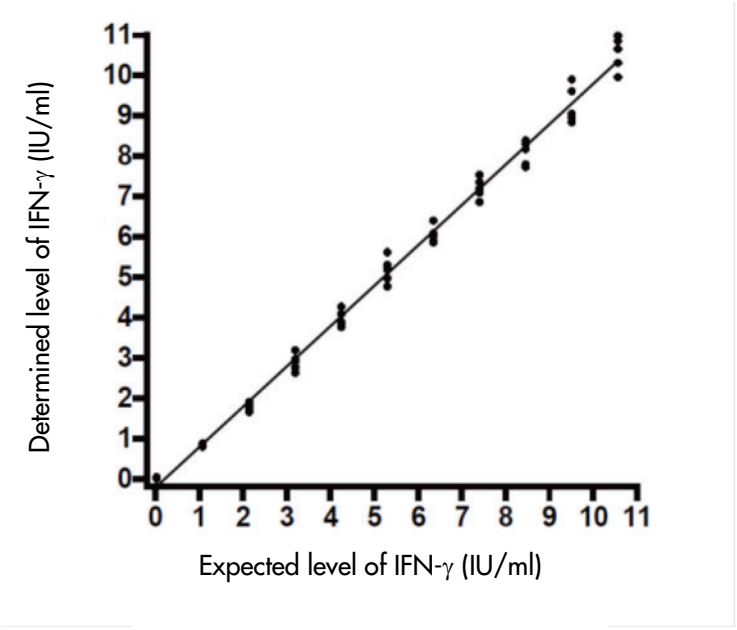


Figure 6. Linearity profile of QFM ELISA determined from testing 5 replicates of 11 plasma samples of known IFN-γ concentrations.

The reproducibility of the QFM assay (Stage 1) was determined using blood samples from 20 healthy subjects. Three different operators, QFM LyoSphere lots, and sets of equipment were assessed. The average coefficient of variation of the IFN-γ response levels determined using the QFM ELISA across all three lots of QFM LyoSpheres and all three conditions tested was 22.22% (95% CI: 17.20–27.25).

The repeatability of the QFM assay (Stage 1) was assessed by measuring the variability of 5–6 repeat QFM LyoSphere blood stimulations within the same donor across 14 subjects. The average coefficient of variation across the 14 subjects tested was 14.7% (95% CI: 10.2–19.2). The %CV of individual subjects was less than 30%.

The reproducibility of the QFM ELISA (Stage 2) was estimated by testing 20 plasma samples with varying IFN-γ concentrations in replicates of 3, in 3 laboratories, on 3 nonconsecutive days, by 3 operators. Thus each sample was tested 27 times, in 9 independent assay runs. One sample was a nil control and had a calculated IFN-γ concentration of 0.08 IU/ml (95% CI: 0.07–0.09). Of the remaining 19 plasma samples, the range of concentrations was 0.33 (95% CI: 0.31–0.34) to 7.7 IU/ml (95% CI: 7.48–7.92).

Within run or intra-assay imprecision was estimated by averaging the %CVs for each test plasma containing IFN-γ from each plate run (n=9) and the imprecision ranged from 4.1 to 9.1% CV. The average within run %CV (±95% CI) was 6.6% ± 0.6%. The average of the zero IFN-γ plasma was14.1% CV.

Total or inter-assay imprecision was determined by comparing the 27 calculated concentrations of IFN-γ for each plasma sample. The interassay imprecision ranged from 6.6 to 12.3% CV. The overall average %CV (±95% CI) was 8.7% ± 0.7%. The zero IFN-γ plasma showed a 26.1% CV. This level of variation is to be expected because the calculated concentration of IFN-γ is low and variation around a low estimate of concentration will be larger than that for higher concentrations.

Technical Information

Clotted plasma samples

Should fibrin clots occur with long-term storage of plasma samples, centrifuge the samples to sediment clotted material and facilitate pipetting of plasma.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Technical Information provided at: www.QuantiFERON.com. For contact information, see the back cover.

ELISA troubleshooting

Nonspecific color development

Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Cross-contamination of ELISA wells	Take care when pipetting and mixing sample to minimize risk.
c) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100x Concentrate are used within 3 months of the reconstitution date.
d) Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.
e) Mixing of plasma in QFM tubes before harvesting	After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.

Low optical density readings for standards

Possible cause	Solution
a) Standard dilution error	Ensure dilutions of the kit standard are prepared correctly as per this package insert.
b) Pipetting error	Ensure pipets are calibrated and used according to manufacturer's instructions.
c) Incubation temperature too low	Incubation of ELISA should be performed at room temperature (17°C to 27°C).

ELISA troubleshooting

d) Incubation time too short	Incubate the plate with the conjugate, standards, and samples for 120 ± 5 minutes. Incubate the Enzyme Substrate Solution on the plate for 30 minutes.
e) Incorrect plate reader filter used	Plate should be read at 450 nm with a reference filter between 620 and 650 nm.
f) Reagents are too cold	All reagents, with the exception of the Conjugate 100x Concentrate, must be brought to room temperature prior to starting the assay. This takes approximately one hour.
g) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100x Concentrate are used within 3 months of their reconstitution date.

High background

Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Incubation temperature too high	Incubation of the ELISA should be performed at room temperature (17°C to 27°C).
c) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted Standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
d) Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.

Nonlinear standard curve and duplicate variability

ELISA troubleshooting

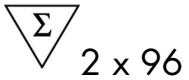
Possible cause	Solution
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Standard dilution error	Ensure dilutions of the standard are prepared correctly as per this package insert.
c) Poor mixing	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
d) Inconsistent pipetting technique or interruption during assay setup	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to starting the assay.

Product information and technical guides are available free of charge from QIAGEN, via your distributor, or by visiting www.QuantiFERON.com.

References

1. Abbas, A.K., Lichtman, A.H., and Pillai, S. (2012) *Cellular and Molecular Immunology*. 7th ed. Philadelphia: Elsevier/Sanders.
2. Fernández-Ruiz, M., Kumar, D., and Humar, A. (2014) Clinical immune-monitoring strategies for predicting infection risk in solid organ transplantation. Clin. Transl. Immunol. **3**, e12.
3. Sood, S. and Testro, A.G. (2014) Immune monitoring post liver transplant. World J. Transplant. **4**, 30.
4. Sood, S. (2014) A novel biomarker of immune function and initial experience in a transplant population. Transpl. J. **97**, e50.

Symbols

	Sufficient for 2 x 96 sample preparations
	Legal manufacturer
	CE-IVD marked symbol
	For in vitro diagnostic use
	Batch code
	Catalog number
	Use by date
	Temperature limitation
	Consult instructions for use
	Do not reuse
	Keep away from sunlight
	Authorized representative in the European Community

Contact Information

For technical assistance and more information, please call toll-free 00800-22-44-6000, see our Technical Support Center at www.qiagen.com/contact or contact one of the QIAGEN Technical Service Departments (see back cover or visit www.qiagen.com).

Abbreviated Test Procedure

Stage 1 — blood incubation

1. Collect patient blood into either a QFM Blood Collection Tube or a lithium heparin blood collection tube. Label tubes with patient details and time of blood collection, then transport to laboratory at ambient temperature within 8 hours of collection.

    a. If blood was collected into lithium heparin blood tube, aliquot 1 ml of blood into a QFM Blood Collection Tube and label the tube with patient details and time of blood collection

2. Add 1 QFM LyoSphere to each QFM Blood Collection Tube containing 1 ml of blood, dissolve LyoSphere, and then incubate tubes as soon as possible (within 8 hours of blood collection) **upright** for 16–24 hours at 37°C.

3. Following incubation, centrifuge tubes for 15 minutes at 2000 to 3000 x *g* (RCF) to separate the plasma and the red cells.

4. After centrifugation, avoid pipetting up and down or mixing the plasma by any means prior to harvesting. At all times, take care not to disturb the material on the surface of the gel.

Stage 2 — IFN-γ ELISA

1. Equilibrate ELISA components, with the exception of the Conjugate 100x Concentrate, to room temperature for at least 60 minutes.

2. Reconstitute the kit standard to 8.0 IU/ml with distilled or deionized water. Prepare 4 standard dilutions.
3. Reconstitute freeze-dried Conjugate 100x Concentrate with distilled or deionized water

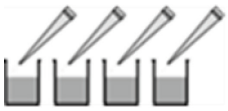
4. Prepare working strength conjugate in Green Diluent and add 50 µl to all wells.

5. Add 50 µl test plasma samples (undiluted, 1:10 and 1:100 dilutions as appropriate) and 50 µl standards to the appropriate wells. Mix using shaker.

6. Incubate for 120 ± 5 minutes at room temperature.

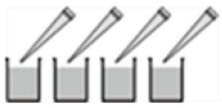
7. Wash wells at least 6 times with 400 µl/well of wash buffer.

8. Add 100 µl Enzyme Substrate Solution to wells. Mix using shaker.

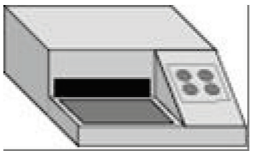


9. Incubate for 30 minutes at room temperature.

10. Add 50 µl Enzyme Stopping Solution to all wells. Mix using shaker.



11. Read results at 450 nm with a 620 to 650 nm reference filter.



12. Analyze results.

Notes

Significant Changes

Significant changes in this edition of the QuantiFERON Monitor® (QFM®) ELISA Package Insert are summarized in the table below:

Section	Page	Change(s)
Materials required but not provided	8	Added Plate Lids
References	34	Removed A comprehensive list of QFM references is located on Gnowee — the QuantiFERON reference library, available at www.gnowee.net.

Notes

Notes

Notes

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